Check List 8(3): 513-515, 2012
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ISSN 1809-127X (available at *www.checklist.org.br*)

Check List
Journal of species lists and distribution

NOTES ON GEOGRAPHIC DISTRIBUTION

Pristimantis citriogaster Duellman, 1992 (Amphibia: Craugastoridae): First record from Ecuador, altitudinal distribution extension with distribution map and phylogram

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ABSTRACT: Using genetic and morphological evidence, we provide the first report of *Pristimantis citriogaster* (Duellman, 1992) from Ecuador (previously only know from the type locality in eastern lowlands of Peru). The new records in Ecuador also represent an altitudinal extension.

The terrestrial craugastorid frogs of the genus *Pristimantis* contain 448 species, making it the most diverse vertebrate genus in the world (Hedges *et al.* 2008). Their diversity center are the tropical Andes and in Ecuador one in three species of anurans belongs to this genus (Hedges *et al.* 2008; Ron *et al.* 2011). *Pristimantis citriogaster* is part of the *P. conspicillatus* species group, which contains 37 species and is distributed principally in northern South America (Hedges *et al.* 2008). It was described by Duellman (1992), the holotype (KU 212277) is an adult female from Cataratas Ahuashiyacu, 06°30' S, 76°20' W, 730 m above sea level, 14 km by road NE from Tarapoto, Departamento de San Martín, Peru. *Pristimantis citriogaster* is only known from its type locality at elevations between 600–800 m, and is categorized as Data Deficient in the UICN Red List (Rodriguez *et al.* 2011).

Herein, we present the first record of *P. citriogaster* from Ecuador based on examination of specimens deposited at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ), from southeast Ecuador (Figure 1). Specimen identification was based on comparisons with morphological characters reported by Lynch and Duellman (1980), Duellman and Pramuk (1999), and Duellman and Lehr (2009). The identification was corroborated by genetic characters from three individuals: QCAZ 17023 (GenBank Accession Number JQ964419; Figure 2), subadult collected in September 2001 by Omar Torres-Carvajal, Jennifer Pramuk, and Lena M. Echelle in General Leonidas Plaza (Limón), Provincia Morona Santiago, 2°57'55.8" S, 78°25'9.4" W at 1094 m; QCAZ 41833 (GenBank Accession Number JQ964421), adult male collected in December, 2007 by David Salazar and María José Endara at Río Napinaza, 6.6 km N from General Leonidas Plaza (Limón), Provincia Morona Santiago, 2°55'22"S, 78°24'26.6" W at 1000 m; and QCAZ 41487 (GenBank Accession Number JQ964420), adult female collected in April, 2009 by Juan M. Guayasamín, Elicio E. Tapia and Silvia Aldás-Alarcón in Las Orquideas, Parroquia Zumi, canton Nangaritza, Provincia Zamora Chichipe, 4°13'44.51" S, 78°39'27.9" W at 874 m.

The Ecuadorian specimens agree with the descriptions of *P. citriogaster* by Duellman (1992), Duellman and Pramuk (1999), and Duellman and Lehr (2009), are characterized by skin on dorsum shagreen without tubercles; indistinct dorsolateral folds; skin on venter smooth; tympanum distinct, round; first finger longer than second; fingers lacking lateral fringes or keels; ulnar tubercles present distally; tubercles absent on heel and tarsus; toes barely webbed basally, lacking lateral fringes or keels; posterior surfaces of the thighs mottled dark brown and cream; flecks on throat and ventral surface of thighs. The characteristic bright yellow belly of *P. citriogaster* in life could not be verified because only photos in dorsal view of live specimens were available. In contrast with Duellman and Pramuk (1999) report of a round palmar tubercle (shared only with *P. zeuctotylus* within the *P. conspicillatus* species group), the Ecuadorian specimens have a bifid palmar tubercle, which would be at the extreme of the variation reported in the original description of *P. citriogaster* by Duellman (1992): "the palmar tubercle could be large,

FIGURE 1. Dorsal view of a subadult *Pristimantis citriogaster* (QCAZ 17023) collected in General Leonidas Plaza (Limón), Provincia Morona Santiago, Ecuador. Photo: Omar Torres-Carvajal.

broadly ovoid; emarginated or slightly bifid distally".

Differences between *Pristimantis citriogaster* and other species of the *P. conspicillatus* species group from the eastern lowlands and foothills of Ecuador are listed in Table 1. In coloration, the venter of *P. citriogaster* has mottling on the throat, laterally on the belly and ventral surfaces of the thighs; *P. condor* has a densely pigmented throat and mottling on the belly and ventral surfaces of the thighs; the venter in *P. lymani* is immaculate except for the presence of dark bars in the margin of the lips and mottling in the posterior surfaces of the thighs. Differences with the other species are listed in Table 1.

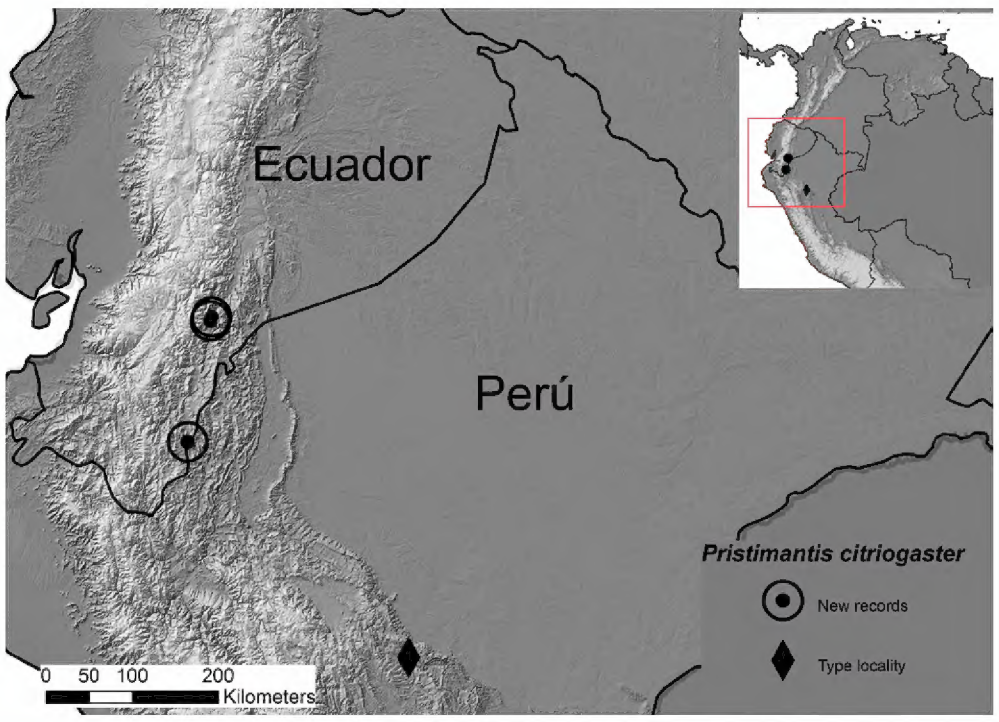

FIGURE 2. Map showing first records of *Pristimantis citriogaster* in Ecuador, based on specimens deposited at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ) and the type locality in Peru extracted from Duellman (1992).

Based on sequences of 582 bp of mitochondrial DNA (16S rRNA gene) obtained using forward primer 16L19 (secuence: AATACCTAACGAACTTAGCGATAGCTGGTT) and reverse primer 16H36E (AAGCTCCAWAGGGTCTTCTCGTC) (Heinicke *et al.,* 2007; Hedges *et al.,* 2008) and a method of PCR amplification under the following conditions: initial denaturation for 5 min at 94 °C for 1 min followed by 45 cycles of 95°C for 20 s, annealing at 52°C for 25 s and extension al 72°C for 1 min and a final extension for 7 min at 72 °C. PCR products were cleaned by ExoSAP digest and sequenced in Macrogen (Macrogen Inc., Seúl, Corea) Secuences were edited and aligned using Geneious Pro 5.4.6 (Drummond *et al.*, 2011) and Mesquite 2.74 (Maddison and Maddison, 2011). The phylogeny was obtained using Bayesian inference as implemented in program MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The Bayesian analysis consisted of two parallel runs of the Metropolis coupled Monte Carlo Markov chain for 1 x 10^6 generations. Each run had four chains. Convergence into a stationary distribution was determined by reaching average standard deviation split frequencies < 0.05 between runs. The first 50% of the sampled generations were discarded as burn-in and the remaining were used to estimate the Bayesian tree, posterior probabilities and other model parameters. The model of sequence evolution was chosen with JModelTest version 0.1.1 (Posada, 2008) using the Akaike Information Criterion with sample size correction as optimality measure.

The phylogeny (Figure 3) shows strong support (Bayesian posterior probability = 0.99) for a clade composed of the Ecuadorian samples of *P. citriogaster* and a paratype of *P. citriogaster* from the type locality in Peru (KU212278) from San Martin, San Martin, Cataratas Ahnashiyacu, 14 km NE Tarapoto (GenBank Accession Number EF493700). The uncorrected *p* genetic distance between the Ecuadorian and Peruvian samples ranges from 3.2% to 3.3%. This genetic distance in 16S rRNA could be indicative of interspecific differences (Fouquet *et al.* 2007; Vieites, *et al.* 2009; Funk *et al.* 2011). Thus, the genetic evidence suggests that the Ecuadorian samples are either *P. citriogaster* or a closely related undescribed species. We assign them to *P. citriogaster* based on the congruence in morphology between our samples and the species descriptions provided by Duellman (1992), Duellman and Pramuk (1999) and Duellman and Lehr (2009).

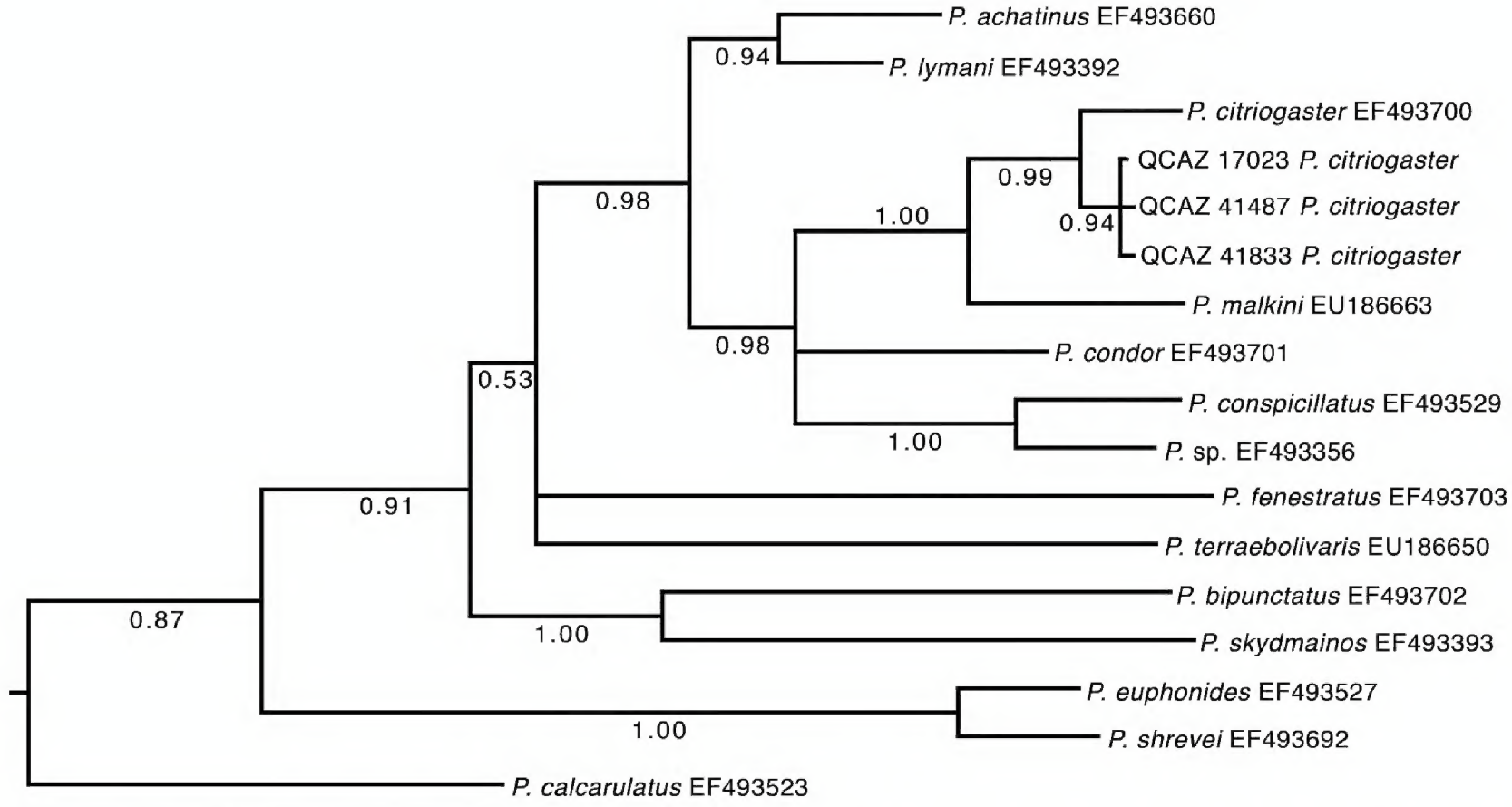

FIGURE 3. Bayesian consensus phylogram depicting relationships among species of the *Pristimantis conspicillatus* species group. Phylogram derived from analysis of 582 bp of mtDNA (gene 16S) under model of evolution GTR + G + I. Numbers next to species names are GenBank accession numbers or QCAZ catalog numbers (for Ecuadorian samples of *P. citriogaster*). Posterior probabilities resulting from Bayesian Markov chain Monte Carlo searches appear below branches.

Table 1. Morphological diagnostic characters and distribution of the species of *Pristimantis conspicillatus* species group from the Amazon Basin in Ecuador. **Pristimantis fenestratus* does not have confirmed records in Ecuador but is probably present according to the potential distribution map shown by Padial and De la Riva (2009); Frost (2011) also reports its presence in southeastern of Ecuador.

SPECIES	ALTITUDINAL RANGE (M)	THROAT AND BELLY	SKIN ON VENTER	POSTERIOR SURFACES OF THE THIGHS	INNER TARSAL FOLD	REFERENCE
Pristimantis citriogaster	600–1094	Cream with brown flecks on throat	Smooth	Mottled dark brown and cream	Present	Duellman, 1992; Duellman and Pramuk, 1999; This work
Pristimantis condor	1300–1975	Densely pigmented throat, mottling on belly	Smooth	Brown with cream or white spots	Short or reduced tubercle	Duellman and Pramuk, 1999; Duellman and Lehr, 2009
Pristimantis conspicillatus	300–1000	Venter white, dusky marks on throat and limbs	Smooth	Dark enclosing white (red in life) spots	Absent	Lynch, 1975; Duellman and Pramuk, 1999
Pristimantis lymani	2000–2500	Cream, immaculate except for dark bars on the margin of lips	Smooth	Black with white spots or reticulations	Long fold	Lynch and Duellman, 1997; Duellman and Lehr, 2009
Pristimantis malkini	< 400	Throat white, venter yellow	Smooth	Black with greenish yellow flecks	Flap-like on distal half of tarsus	Lynch, 1980; Duellman and Pramuk, 1999
Pristimantis metabates	525–860	Dark flecks on throat	Smooth	Dark brown with cream to red spots	Bearing distinct fold distally	Duellman and Pramuk, 1999; Cisneros-Heredia *et al.* 2009
Pristimantis skydmainos	250–750	Venter cream with brown flecks on throat	Weakly areolate	Dark with minute cream flecks	Bearing distinct fold distally	Flores and Rodriguez, 1997; Cisneros-Heredia, 2006; Duellman and Lehr, 2009
*Pristimantis fenestratus**	Not confirmed in Ecuador	Pale cream with brown spots or mottling on throat	Smooth	Yellowish, pale grayish, or pale reddish (same as dorsal color)	Low fold distally	Duellman and Lehr, 2009. Frost, 2011

The Ecuadorian records increase the latitudinal distribution range in 452 km northwest from the type locality and extends the altitudinal upper limit to 1094 m above sea level. This new record increases the diversity of *Pristimantis* to 109 species in Ecuador and confirms the continuity of lowland *Pristimantis* communities between northern Peru and southern Ecuador (Yánez-Muñoz *et al.* 2012).

Acknowledgments: This work had the support of the project "Inventario y Caracterización Genética y Morfológica de la Diversidad de Anfibios, Aves y Reptiles de los Andes del Ecuador" (PIC-08-0000470) granted by SENESCYT, Secretaría Nacional de Ciencia y Tecnología del Ecuador and executed by Museo de Zoología (QCAZ) de la Pontificia Universidad Católica del Ecuador. Ministerio de Ambiente of Ecuador and Dirección General Forestal y Fauna Silvestre (DGFFS) issued collection permits (008-09 IC-FAU-DNB/MA). We thank Omar Torres-Carvajal, Jennifer B. Pramuk, Lena M. Echelle, David Salazar, María José Endara, Juan M. Guayasamín, Elicio E. Tapia, and Silvia Aldás-Alarcón for specimen collection. Teresa Camacho Badani thanks to Organization of American States Scholarships for supporting her graduate studies at the Masters program in Conservation Biology at Pontificia Universidad Católica del Ecuador. Mario Yanez-Muñoz thanks to the Unidad ABC of the I. Municipio de Quito for partly supporting his participation at the same Masters program.

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Received: February 2012
Accepted: April 2012
Published online: June 2012
Editorial responsibility: Raúl Maneyro